

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

LONZA BIOLOGICS PLC.
Legal Department
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GRANDE BRETAGNE

RECEIVED

19 JUN 2006

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY

(PCT Rule 71.1)

EINGEGANGEN

21. Juni 2006

Date of mailing
(day/month/year)

13.06.2006

Applicant's or agent's file reference
LBP1005PC00

Erl.....

IMPORTANT NOTIFICATION

International application No.
PCT/EP2005/002538

International filing date (day/month/year)
10.03.2005

Priority date (day/month/year)
10.03.2004

Applicant
LONZA LTD. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international
preliminary examining authority:



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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference LBP1005PC00	FOR FURTHER ACTION See Form PCT/PEA/416	
International application No. PCT/EP2005/002538	International filing date (day/month/year) 10.03.2005	Priority date (day/month/year) 10.03.2004
International Patent Classification (IPC) or national classification and IPC INV. C07K16/00		
Applicant LONZA LTD. et al.		
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 4 sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>		
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the report</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>		
Date of submission of the demand 01.10.2005	Date of completion of this report 13.06.2006	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Armandola, E Telephone No. +49 89 2399-7493 	

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

 International application No.
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Box No. I Basis of the report

 1. With regard to the **language**, this report is based on

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
☐ international search (under Rules 12.3(a) and 23.1(b))
☐ publication of the international application (under Rule 12.4(a))
☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

 2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*
Description, Pages

1-44 as originally filed

Claims, Numbers

1-18 received on 09.02.2006 with letter of 07.02.2006

Drawings, Sheets

1/1 as originally filed

- ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

 3. ☒ The amendments have resulted in the cancellation of:

- ☐ the description, pages
☒ the claims, Nos. 18-47, 49-57
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to sequence listing (*specify*):

 4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-18
	No: Claims	
Inventive step (IS)	Yes: Claims	1-18
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-18
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

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Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ on paper
 - ☒ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed
 - ☐ filed together with the international application in electronic form
 - ☒ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment* on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
 - * *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

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Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

Reference is made to the following documents:

- D1: SWENNEN DOMINIQUE ET AL: "Secretion of active anti-Ras single-chain Fv antibody by the yeasts *Yarrowia lipolytica* and *Kluyveromyces lactis*" MICROBIOLOGY (READING), vol. 148, no. 1, January 2002 (2002-01), pages 41-50, XP002350347 ISSN: 1350-0872
- D2: WONG MICHAEL J ET AL: "Processing of human factor I in COS-1 cells co-transfected with factor I and paired basic amino acid cleaving enzyme (PACE) cDNA" MOLECULAR IMMUNOLOGY, vol. 32, no. 5, 1995, pages 379-387, XP002350310 ISSN: 0161-5890
- D3: EP-A-1 099 758 (GENENTECH INC) 16 May 2001 (2001-05-16)
- D4: SANTOS A D ET AL: "GENERATION AND CHARACTERIZATION OF A SINGLE GENE-ENCODED SINGLE-CHAIN-TETRAVALENT ANTITUMOR ANTIBODY" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 5, no. 10, October 1999 (1999-10), pages 3118S-3123S, XP000929841 ISSN: 1078-0432 cited in the application
- D5: RIDDER R ET AL: "GENERATION OF RABBIT MONOCLONAL ANTIBODY FRAGMENTS FROM A COMBINATORIAL PHAGE DISPLAY LIBRARY AND THEIR PRODUCTION IN THE YEAST *PICHA PASTORIS*" BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 13, no. 3, March 1995 (1995-03), pages 255-260, XP002008019 ISSN: 0733-222X
- D6: LEE JEEWON ET AL: "Novel secretion system of recombinant *Saccharomyces cerevisiae* using an N-terminus residue of human IL-1 β as secretion enhancer" BIOTECHNOLOGY PROGRESS, vol. 15, no. 5, 1999, pages 884-890, XP002211009 ISSN: 8756-7938
- D7: WHITTLE N ET AL: "EXPRESSION IN COS CELLS OF A MOUSE - HUMAN CHIMAERIC B72.3 ANTIBODY" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 1, no. 6, 1 December 1987 (1987-12-01), pages 499-505, XP000005250 ISSN: 0269-2139 cited in the application

Novelty and Inventive step (Art. 33(2), 33(3) PCT)

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Document D1 discloses a single chain anti-p20 ras antibody comprising only the variable regions, expressed in yeast using the Kex2 protease processing sequence that links the scFv to a reporter secretory sequence (page 45, Figs. 1 and 2).

Document D4 discloses the production of a single gene-encoded single-chain tetravalent antibody. The heavy and light chains comprise VH, CH2, CH3 or VL, CL domains, respectively. The heavy and the light chain are joined by a linker. No protease cleavage site is comprised in the linker.

Document D7 discloses the production of the chimeric human-mouse mAb B72.3 in CHO cells by transfection of separate constructs encoding heavy and light chains. No linker between heavy and light chain nor the presence of a protease site are described.

In view of these documents the subject-matter of claims 1-18 can be considered novel.

The claims can also be considered to entail an inventive step.

Document D7 can be considered the closest prior art. The difference between D7 and the application is the construct transfected in the host cells that comprises heavy and light chains joined by a linker that is then cleaved by a protease. The problem to be solved can be defined as that of improving the production of a tetrameric MAb in transfected cell by increasing the efficiency of correct chain pairing.

Such a problem has been recognized in the prior art and solved by introducing a linker between heavy and light chain, thus bringing the two chains in close proximity (higher pairing chances). Such an approach is, for example, described in D4. In this document, however, the heavy and light chains remain joined by the linker.

The production of multichain proteins by expressing the chains joined by a linker that is then cleaved has been described, for example, in D2 and D3.

Document D2 discloses the recombinant expression in CHO-K1 or COS-1 cells of human factor I as a fusion protein wherein the heavy and the light chains are linked by a dibasic linker that can be cleaved upon cotransfection of the cDNA encoding the protease PACE.

Document D3 discloses the expression of prorelaxin in CHO cells using two cleavage sites for furin-like proteins, a dibasic and a tetrabasic site.

The employment of this strategy, however, is neither described nor hinted at for immunoglobulins.

In view of the prior art the skilled person would not have arrived at the claimed method

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with reasonable expectation of success without the use of inventive skills.

Amended Claims /IPEA:

1. Method for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which immunoglobuline molecule when secreted from a vertebrate host cell comprises at least a first and a second polypeptide chain wherein the first polypeptide is an Ig-Light Chain (L) comprising at least a VL and a CL domain and in that the second polypeptide is an Ig-Heavy Chain (H) comprising at least a a VH, CH2 and CH3 domain and a hinge domain, comprising the steps of
 - a. expressing in a vertebrate host cell having Golgi-only or late-Golgi-only resident furin family endoprotease activity an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least the said first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity and wherein the fusion polypeptide comprises the sequences of said first and second polypeptide separated by a linker and
 - b. having the fusion polypeptide cleaved in the cells by the furin family endoprotease activity into the first and second polypeptide chains and
 - c. harvesting the secreted immunoglobuline.
2. Method according to claim 1, characterized in that the Ig molecule comprises a CH1 domain.
3. Method according to claim 1, characterized in that the Light and Heavy Chain are separated by a linker, in that the fusion polypeptide comprises at least two cleavage sites for the furin family endoprotease activity and in that the linker is cleaved off from both Heavy and Light Chain by the furin family endoprotease activity by means of said two cleavage sites.

4. Method according to claim 1, characterized in that the furin family endoprotease activity is an activity naturally present in that host cell line.
5. Method according to claim 1, characterized in that the host cell is devoid of furin family endoprotease activity in the endoplasmic reticulum.
6. Method according to claim 1, characterized in that the furin family endoprotease activity is furin endoprotease or lymphoma proprotein convertase or a functional variant thereof.
7. Method according to claim 1, characterized in that the furin family endoprotease activity is a constitutive endoprotease activity.
8. Method according to claim 1, characterized in that the host cell line is a mammalian cell line, preferably is a CHO cell.
9. Method according to claim 8, characterized in that the host cell has at least one recombinant furin family endoprotease activity which is a homologously expressed mammalian furin family endoprotease naturally present in that host cell line which further is an constitutive furin family endoprotease or furin family endoprotease belonging to constitutive secretion, in this way achieving an elevated expression level of the natural gene product in its native host cell environment.
10. Method according to claim 15, characterized in that the mammalian host cell line are CHO cells.
11. Method according to claim 1, characterized in that the cleavage sites is a contiguous tetrapeptide sequence comprising at least three basic residues selected from the group consisting of arginine and lysine.

12. Method according to claim 11, characterized in that the tetrapeptid sequence comprises four basic residues selected from the group consisting of arginine and lysine.
13. Method according to claim 1, characterized in that the linker is a non-naturally occurring amino acid sequence.
14. Method according to claim 1 or 13, characterized in that the linker comprises at least 20 amino acids.
15. Method according to claim 14, characterized in the linker comprises one or several oligomers consisting of only glycine and either serine, threonine or both.
16. Method according to claim 15, characterized in that the linker consists of one or several oligomers consisting of only glycine and either serine, threonine or both.
17. Method according to claim 15 or 16, characterized in that the linker comprises at least >60% glycine residues.
18. Method for producing an immunoglobuline having Fc receptor activity and/or complement activation activity which immunoglobuline molecule when secreted from a vertebrate host cell comprises at least a first and a second polypeptide chain wherein the first polypeptide is an Ig-Light Chain (L) comprising at least a VL and a CL domain and in that the second polypeptide is an Ig-Heavy Chain (H) comprising at least a a-VH, CH2 and CH3 domain and a hinge domain, comprising the steps of
 - a. expressing in a vertebrate host cell having Golgi-only or late-Golgi-only resident subtilisin/kexin family endoprotease activity an fusion polypeptide comprising a secretion targetting sequence directing the polypeptide to the secretory pathway and further comprising at least

the first and second polypeptide sequences and at least one cleavage site for the said endoprotease activity and wherein the fusion polypeptide comprises the sequences of said first and second polypeptide separated by a linker, and

- b. having the fusion polypeptide cleaved in the cells by the subtilisin/kexin family endoprotease activity into the first and second polypeptide chains and
- c. harvesting the secreted immunoglobuline.